Phage-Host Interactions: *In Vitro* Generated *E. coli* Phage Resistance

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Abstract

Avian pathogenic *Escherichia coli* (APEC) is one of the most important bacterial pathogens affecting poultry worldwide [1]. In recent years, there has been a renewed interest in the therapeutic use of bacteriophages (phages) as alternative or supportive to existing antibiotics [2]. However, despite promising *in vitro* data successful *in vivo* therapeutic activity is not always guaranteed [3]. Therefore, a better understanding of the phage-bacterium interactions and the underlying mechanisms is essential for the successful development of a phage therapy.

Here, we investigated the interactions of the APEC O1:H7 ST95 strain, AM621, and the well-characterized lytic *Myoviridae* phage, *Escherichia* phage vB_EcoM-P10, at different multiplicity of infection (MOI). These interactions led to 109 spontaneous *in vitro* generated phage-resistant mutants of the APEC strain. These mutants were obtained using the agar plate or the secondary culture method [4]. Resistance mechanisms that occurred in the bacterial mutants were investigated through analysis of whole genome sequencing (WGS) data. The analysis revealed 41 mutants with single-nucleotide polymorphisms (SNPs) in their core genome. In 32 of the mutants a single SNP was detected while two SNPs were identified in nine mutants. In total, 34 unique SNPs were detected. The SNPs resulted in nonsense (n=5), missense (n=19), or synonymous mutation (n=6). The remaining four SNPs were in non-coding regions. Affected genes included outer membrane protein (omp) A, lipopolysaccharide (LPS)-related genes, phosphate acetyltransferases, and genes with unknown function. In 42 strains, including 18 strains with SNP(s), gene losses were detected. Overall, this concerned 20 different genes, most of which had unknown function. One or more genes were associated with antibiotic resistance, omp, or the O-antigen. Moreover, in three strains we identified a spacer in the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) that matched the invading phage genome. For 46 mutant strains we were not able to detect any genomic changes that could differentiate them from the WT strain. The reason for this remains obscure and will require further investigation.

In conclusion, this study provides new insights into the phage-host interaction and phage resistance in APEC. However, to fully understand the complexity of the phage-host interaction, the underlying mechanisms need to be further deciphered.

References

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